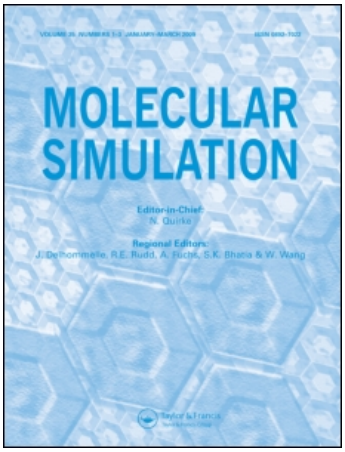

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Molecular Simulation to Aid in the Understanding of the Aβ(1-42) Peptide of Alzheimer's Disease

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MOLECULAR SIMULATION TO AID IN THE UNDERSTANDING OF THE Aβ(1-42) PEPTIDE OF ALZHEIMER's DISEASE

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The Aβ(1-42) peptide of Alzheimer's disease was studied by molecular modeling. The coordinates of the peptide were experimentally generated from solution-NMR spectroscopy, and the conformations were energy minimized using a combination of connectivity-based iterative partial equalization of orbital electronegativity with the MM+ force field.

There is a central folded domain in the Aβ peptide. This part is an apolar α-helix. The remaining residues form β-sheets. Aggregation requires that β-sheets interact by noncovalent bonding forces. The unsoluble, aggregated complexes are energetically stable and have ordered structures.

A perspective in drug research is to design compounds that inhibit the hydrophobic cores of the individual Aβ peptides, blocking so the associations between the β-strains.

Keywords: Molecular simulation; Morbus Alzheimer; Amyloid; Aggregation of peptides; Aβ peptides; α-helix; β-sheet; Conformational transitions; Hydrophobic cores of β-sheets; Drug design

1. INTRODUCTION

Alzheimer's disease (AD) is a chronic, neurodegenerative disorder which is characterized by pathological brain lesions composed of amyloid deposition. The major protein constituent of the deposits is the so-called amyloid β-peptide (Aβ) which is derived from proteolysis of a large (653 to 770

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amino acids) transmembrane amyloid precursor protein (APP) by an endosomal/lysosomal processing pathway. Several variants of the naturally occuring Aβ's differ only at the C-terminus (amino acid residues 1–39, 1–40, 1–42, 1–43). It appears that the Aβ(1-40) and Aβ(1-42) are the predominant proteins in neuritic plaque while Aβ(1-43) is present as a minor component, and Aβ(1-39) is a predominant component in cerebrovascular deposits [16, 19]. It was demonstrated that freshly prepared random-coil conformation of Aβ(1-40) and Aβ(1-42) are nontoxic or less toxic, while an enhanced neurotoxicity is observed after inducing an aging of a β-sheet conformation in suitable chemical solution conditions [22].

In this paper, a model system to aid in understanding of conformations of the Aβ(1-42) peptide and its simulated aggregation to dimeric and tetrameric peptides is presented.

2. METHOD

The coordinates of the Aβ(1-40) peptide were generated from solution NMR data (25 degrees Celsius) and downloaded from the Brookhaven Protein Data Bank (1AML file). The 1AML file included six low-energy conformations. To get the Aβ(1-42) peptide, the two missing amino acids Ile41 and Ala42 were added by a sequence editor. Geometry optimization was used to improve the geometry data and to get the most stable conformers due to three reasons: (i) the addition of the two amino acids Ile41 and Ala42, (ii) because NMR spectroscopy may produce a considerable variability of bond lengths, bond angles, and torsion angles due to experimental errors, and (iii) the resulting NMR-solution structures led to six low-energy conformations but not to a hypothetical global-minimum energy conformation.

The conformations were initially energy minimized using the MM+ force field without an electrostatic term. The MM+ empirical potential (force field) is an improved MM2/MM3 version [1, 26]. The whole MM+ procedure was repeated with electrostatic parameters of the connectivity-based iterative partial equalization of orbital electronegativity (Gasteiger method, [10]). After including the partial charges, the resulting conformation contained the molecular electrostatic potential and electrostatic energies. Correlation-gradient geometry optimization [8] was then achieved. The structures were refined using a conjugate gradient minimizer (Fletcher-Reeves modification of the Polak-Ribière method). Convergence was obtained when the gradient root mean square *RMS* was $RMS < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Polar and Apolar Domains

The primary structure of the complete Aβ(1-43) peptide is given in Table I. The Aβ(1-42) peptide was chosen as model agent due to various reasons:

TABLE I Sequence of the Aβ(1-43) molecule. The NH2 and COOH terminal moieties are in 1 and 43 position, respectively. If the studied Aβ(1-42) peptide is considered, the N- and C-terminal moieties are in positions 1 and 42, respectively

Position	*Acid*
1	Asp
2	Ala
3	Glu
4	Phe
5	Arg
6	His
7	Asp
8	Ser
9	Gly
10	Tyr
11	Glu
12	Val
13	His
14	His
15	GlN
16	Lys
17	Leu
18	Val
19	Phe
20	Phe
21	Ala
22	Glu
23	Asp
24	Val
25	Gly
26	Ser
27	Trp
28	Lys
29	Gly
30	Ala
31	Ile
32	Ile
33	Gly
34	Leu
35	Met
36	Val
37	Gly
38	Gly
39	Val
40	Val
41	Ile
42	Ala
43	Thr

it is – beside Aβ(1-40) – the predominant peptide in neuritic plaques. However, the Aβ(1-42) peptide aggregates more easily so that it is virtually impossible to assess experimentally chemical purity above the 70% level [30]. This implies that molecular simulation is suitable for conformational studies.

A categorization in various classes of amino acids is possible. Class A included those amino acids that are hydrophobic, nonpolar, and small-sized (Gly, Ala); class B contained hydrophobic and polar amino acids with both hydrogen acceptor and donor properties (Ser, G1N); class C included basic, charged, and polar amino acids with hydrogen donor properties (Arg, His, Lys); class D included acid, charged, and polar amino acids with hydrogen acceptor properties (Asp, Glu, and Met if it is assumed that Met has a sulfonium ion in solution), and class E contained nonpolar, hydrophobic amino acids with medium and large size (Val, Leu, Phe, Trp).

There are polar areas (such as Ser8, Gly9, Gly10; Gly25, Ser26, AsN27; Gly37, Gly38) and ionized residues (negatively charged: Asp1, Glu3, Asp7, Glu11, Glu22, Asp23; positively charged: Arg5, His6, His13, His14, Lys16, Lys28). The presence of these moieties is one of the conditions that the peptide is able to interact with bulk water and membrane components, such as lecithin, sphingomyelines, or cerebrosides [27]. On the other hand, the polar areas are also able to interact specifically with Aβ peptide receptors (see [30]).

Furthermore, nonpolar areas can also be observed. The determination of the hydrophobicity index of the Aβ(1-42) peptide and of the distribution coefficients (octanol/water) has shown that the (i) monomeric, purified Aβ peptides are soluble in water, (ii) degree of solubility depends on the *p*H, (iii) C-terminal amino acids are less hydrophilic than the amino acids at the N-terminus, and (iv) hydrophilicity of the N-terminus depends more strongly on the *p*H of the tissues than the hydrophilicity of the C-terminus.

A first hydrophobic core was found at the middle domain of the Aβ(1-42) peptide, and a second core from Lys28 to Val40 (Figs. 3 and 5).

Taking the previous results together, both specificity of polar interactions and nonspecificity by apolar interactions, seem to be important for the establishment of fibrillar aggregation.

3.2. Monomeric Aβ(1-42) Peptide Conformations

Six low-energy conformations were obtained by NMR-solution spectroscopy. Therefore, the Gasteiger-MM+ geometry-optimized conformation of the Aβ(1-42) peptide was determined (Fig. 1).

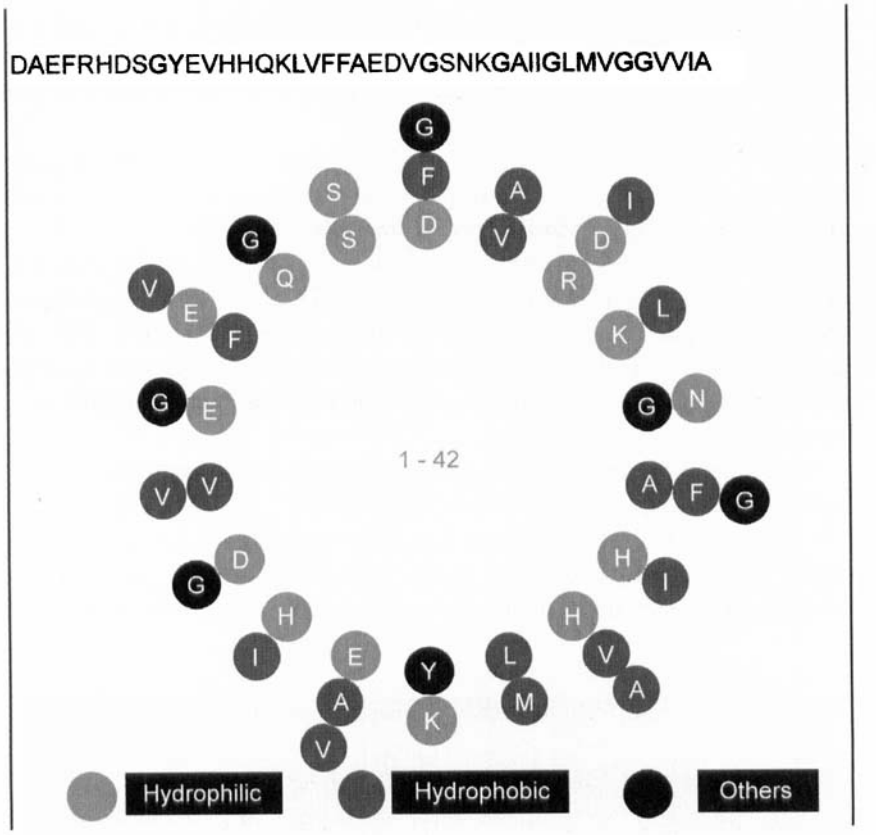

FIGURE 1 Gasteiger-MM + geometry-optimized conformation of the Aβ(1-42) peptide. The input coordinates of the spatial structure was determined by NMR solution spectroscopy. The optimized molecule can be downloaded from the Internet in order to get a visual impression of the three-dimensional structure (address see text). Colors: green, carbon; red, oxygen; white, hydrogen; blue, nitrogen; yellow, sulfur. The yellow-marked area in the middle is the lipophilic, α-helical, folder region (Leu17 to Ala21) which allows to build the two antiparallel β-sheets of the remaining amino acids. It is the potential receptor site of β-sheet-blocking peptidomimetics. (See Color Plate I).

It was suggested that a structural transition from an α-helix into a β-sheet is believed to be a prerequisite for filament formation [3, 11, 15, 22]. Therefore, it would be useful to design an idealized α-helix and β-sheet conformation of the Aβ(1-42) peptide. A random number generator was used to assign the angles for each residue according to a Gaussian probability distribution, so that the secondary structure was a random-coil conformation. Reinitiation of an ordered structure was then achieved by addition of ordered-structure-enforcing local constraints. The constraints

were taken as a function of the degree of structural similarity of a number of known structures and conformations. The standard geometries of an α-helix and β-sheet were defined according to literature [4, 18, 29]. Figures 2–4 give an impression of the conformations.

The α-helix has the lowest total energy, mainly due to the highly negative Coulomb energy. Structural transitions from an α-helix into a random-coil and the β-sheet conformation require the input of energy, therefore. Suitable solvent conditions for structural transitions were described elsewhere [3, 6, 11, 22]. For example, at low *p*H values where carboxylate residues are protonated, the α-helix is uncharged and lipid-soluble. If the *p*H increases above 6, deprotonation appears which facilitates unwinding of the α-helix [6]. The polar and apolar amino acids of an α-helix are shown in Figure 3.

However, neither an idealized α-helix nor a pure β-sheet are able to satisfy the theoretical requirements of an establishment of fibrillar aggregations [15]. It must be required that there is at least a nonpolar folding part in the middle of the peptide which is highly ordered (α-helical type, Lys16 to Ala21), and a large area of a β-sheet conformation which is lipophilic (Lys28

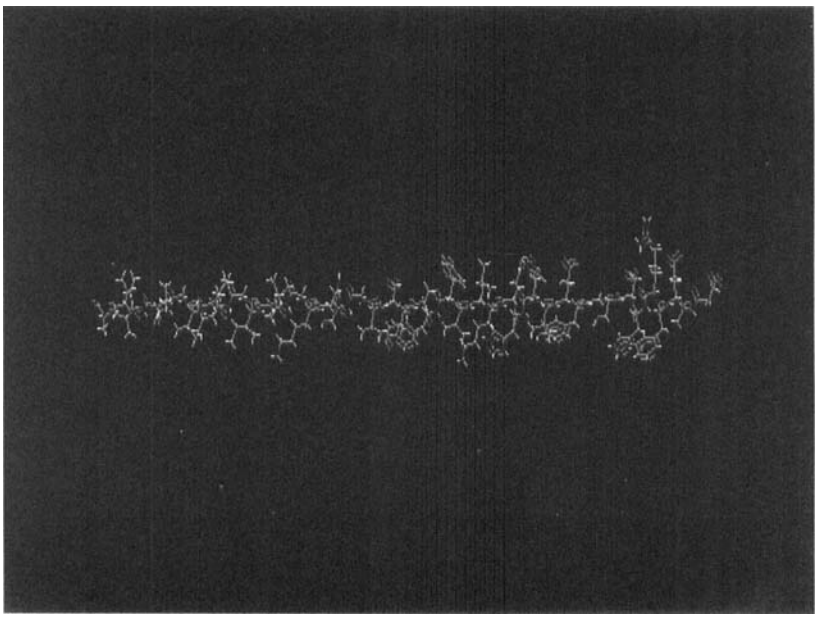

FIGURE 2 Typical picture of a Gasteiger-MM+ geometry optimized conformation of the α-helix of Aβ(1-42). (See Color Plate II).

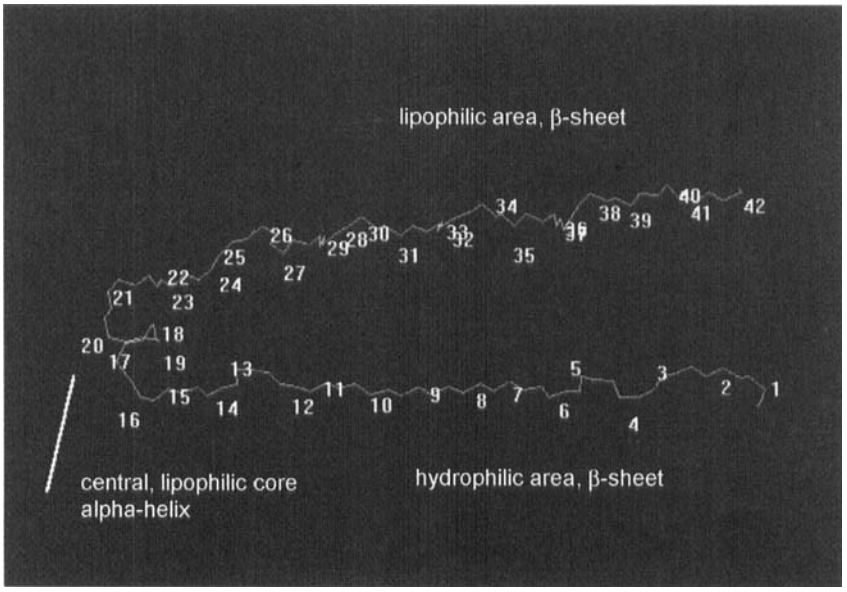

FIGURE 3 Helical wheel with polar and apolar amino acids. (See Color Plate III).

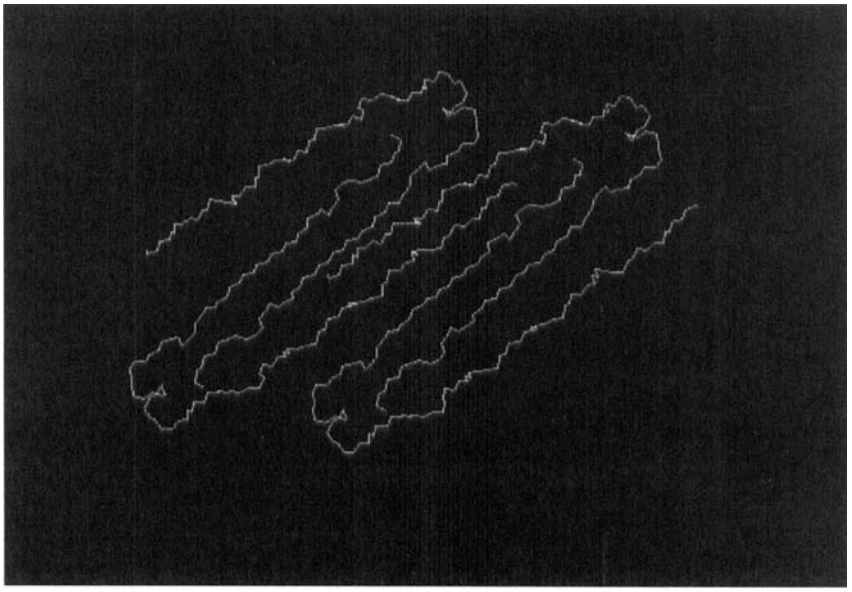

FIGURE 4 Typical picture of a Gasteiger-MM + geometry optimized conformation of the β-sheet of Aβ(1-42). (See Color Plate IV).

to Val40). The first conditions makes the molecule relatively stable by intramolecular hydrogen-bonding forces, the second conditions allows aggregation of lipophilic residues by intermolecular noncovalent interactions. Furthermore, it must be hypothesized that there are polar, conformationally flexible residues that can accommodate possible receptor areas.

The proposed idealized and geometry-optimized structure is a loop-like molecule (Fig. 5). Its central domain, an α-helical part, is nonpolar and stabilized by hydrogen bonds. At least two strong hydrogen-bonding forces exist (≤ 3.2 Å, ≥ 120 deg): between the NH of the peptide bond of Ala21-Glu22, and the CO of the peptide bond of Val18-and Phe19; and between the NH of the peptide bond of Val18-Phe19, and the CO of the peptide bond of G1N15-Lys16. The model shows the role of the first lipophilic core located in the middle of the peptide. The second lipophilic core is at the end of the peptide (Fig. 5). The two domains have a strong antigentic potential (*Mol. Simul.*, in press).

It appears that the two cores have an amyloidogenic potential; the first core allows to form an α-helix (intramolecular stabilization by folding), the

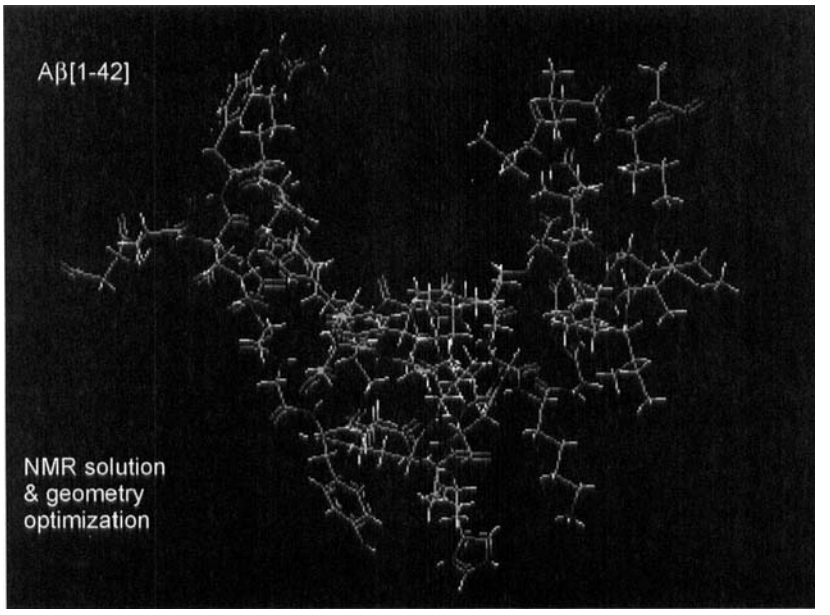

FIGURE 5 Typical backbone structure (without amino acid side chains) of a Gasteiger-MM+ geometry optimized conformation of the loop with a short α-helical part in the middle of the Aβ(1-42) chain and long β-sheets. (See Color Plate V).

second core (β-sheet) enables the molecule to form intermolecular bonds with other β-sheets.

The model agrees with the known rule that β-sheets can provide the key elements in protein-protein (and protein-lipid, protein-DNA, protein-RNA) recognition and subsequent signal transduction [21, 23], and exhibit cooperative two-state folding-unfolding transitions [14].

The charged, solvent-exposed areas are potential sites of an interaction with large polyelectrolytes. A more specific cellular recognition site for Aβ peptides has been proposed quite recently, it is a thirteen amino acid region of the C1q component of the complement cascade (see [30]). At $p\mathrm{H} > 5.42$, the number of negative net charges of the Aβ peptide increases. Therefore, it must be supposed that the hypothetical Aβ receptor is positively charged at physiological *p*H. The proposed structure directs the attention to a prediction of circular dichroism spectra [20] of the loop. The predicted spectrum is closely related with experimentally observed spectra of neurotoxic Aβ(1-42) peptides [22].

3.3. Oligomeric Aβ(1-42) Peptide Conformations

To simulate the process of crystallization, two and four geometry-optimized loops were aggregated by molecular simulation. The structures were positioned in opposite directions related to the N- and C-terminal moieties. The result of four aggregated loops is illustrated in Figure 6.

Intra- and intermolecular interactions of the filamentous-like aggregates are mainly based on weak noncovalent $\pi-\pi$, $\sigma-\sigma$, and $\pi-\sigma$ bonding forces, such as van-der-Waals interactions as sum of dispersion and repulsion energies, hydrogen bonds, London forces, hydrophobic bonds, and pure electrostatic interactions which are solvent-dependent [12]. Among these forces, lipophilic, long-ranged forces [7] play probably a role. The hypothesis is supported by the fact that a maximum aggregation of Aβ peptides to unsoluble amyloids occurred at *p*H values which are near the predicted isoelectric point (IP) of $\mathrm{IP} = 5.42$. This indicates that a net zero charge of the peptide favors aggregation at this *p*H, that is, noncovalent interactions play indeed a dominant role. At higher *p*H, noncovalent bonds are not the only type of interaction, however. The specificity to the Aβ peptide receptor is probably based on polar bonding forces that are much more strong than the inter- and intramolecular lipophilic bonding forces. The insoluble aggregated complexes are very stable, compared to the results of energy calculations obtained by a single loop. The backbone of the tetramer is highly ordered in structure. Therefore, it might be hypothesized

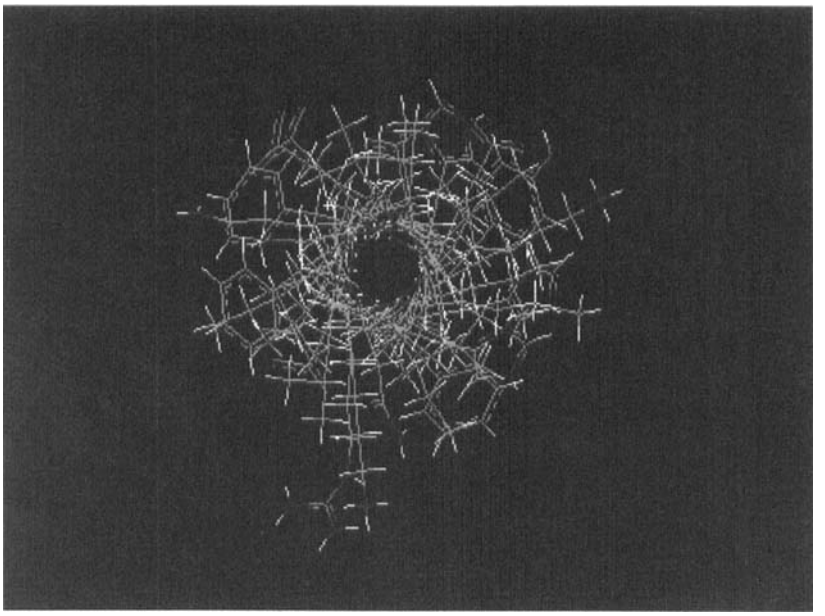

FIGURE 6 Example of the backbone structure (without amino acid side chains) the Gasteiger-MM+ geometry optimized conformation of the tetrameric Aβ(1-42) peptide (four aggregated loops). (See Color Plate VI).

that the tetramer will be more stable in preparations containing high concentrations of other polyelectrolytes. This agrees with the finding that Aβ peptides insert into lipid bilayers and into the membrane hydrocarbon core of liposomes [27]. These binding processes may be facilitated by the presence of charged membrane surfaces [21, 31], and the resulting complex may act as a seed for the growth of larger oligomers. The macromulecular complex may be considered as a "superantigen" [32].

4. CONCLUSIONS AND FUTURE PERSPECTIVE

Three functional areas of the Aβ(1-42) peptide were found: (i) a lipophilic region in the middle of the peptide, (ii) a second core at the end (Lys28 to Val40), and (iii) charged, solvent-exposed areas.

Using molecule coordinates found experimentally by NMR-solution spectroscopy, subsequent Gasteiger-MM+ geometry optimization led to the result that the central, first lipophilic core has an α-helical structure which is stabilized by intramolecular hydrogen bonding forces. The result is

a loop-like molecule. The second lipophilic core has a β-sheet structure, is able to form noncovalent forces with other β-sheets of Aβ peptides where the β-strands run in an unequal amide-to-carbonyl direction. The insoluble aggregates are highly stable and ordered. The charged residues are potential sites for a cross-linking with membrane-bound receptors (from macromolecules like cerebrosides, the enzyme acetylcholinesterase, to more specifically acting receptors such as the C1q component of the complement cascade).

A perspective in drug research could be the development of drugs that bind to individual β-sheets by noncovalent interactions between the lipophilic cores, blocking so associations between the individual Aβ peptides. Quite recently, it was shown that β-sheet blockers containing amino acids in a typical α-helical or random-coil conformation, are bound to the (1–40) and Aβ(1-42) peptide, inhibiting thus amyloid formation [9, 13, 24, 25, 28]. However, based on the proposed model it can be concluded that the β-sheet breaking effect is the final event; the proper effect is the inhibition of the folding of the short α-helical, lipophilic region of the monomeric peptide.

Results on the formation of a stable, neurotoxic acetylcholinesterase-Aβ-peptide complex in the brain [2], a selective binding of the Aβ(1-42) peptide with the nicotinic acetylcholine receptor [33], and the reduction of acetylcholine synthesis by Aβ peptides [17], link the apparently different hypotheses of the generation of Alzheimer's disease. This link can also be made for the free-radical peptide hypothesis [5].

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